Angiotensin-converting enzyme and the processing of regulatory peptides in insects

R.Elwyn Isaac, Department of Biology, University of Leeds, Leeds LS2 9JT, UK

Mammalian angiotensin-converting enzyme (ACE) is a dipeptidyl carboxypeptidase best known for its role in the biosynthesis of the potent vasoconstrictor angiotensin II from angiotensin I and the inactivation of the vasoconstrictor, bradykinin (BK). ACE can also act *in vitro* as an endopeptidase hydrolysing C-terminally amidated peptides (e.g. [Leu^5]enkephalinamide, [Met^5]enkephalinamide, substance P,. cholecystokinin and LH-RH). Somatic ACE is a two-domain protein (the result of a gene duplication event) with each domain containing an active site. ACE is not restricted to the vascular endothelium and is found in many mammalian tissues including the brain, heart, testis, semen, cerebrospinal fluid, blood and lymph. However, the physiological role of ACE in these tissues is not known.

We have recently shown that ACE is of ancient evolutionary origin by characterising a highly homologous enzyme from insects. ACE purified from adult houseflies (*Musca domestica*) has an Mr of 67,000, is inhibited by mammalian ACE inhibitors captopril and fosinoprilat, and removes dipeptides from the C-terminus of angiotensin I, bradykinin, [Leu^5]enkephalin and [Met^5]enkephalin. Housefly ACE also attacks C-terminally amidated peptides (e.g. substance P, [Leu^5]enkephalinamide, [Met^5]enkephalinamide and LH-RH) releasing di- and tri-peptide amides. Most of the insect bioactive peptides that have been characterised possess an amidated C-terminus and many of these are susceptible to hydrolysis by insect ACE (e.g. leuocokinins, locustatachykinins). The structure of insect ACE has been revealed by the cloning and functional expression of an ACE cDNA (AnCE) from a *Drosophila melanogaster* cDNA library. *AnCE* codes for a single-domain 615 amino acid protein with a high level of homology to each of the domains of mammalian ACEs, especially around the active site region, but differs from the major forms of mammalian ACE in that it has a single-domain and is a soluble secreted protein.

ACE is present at all stages of development of *Drosophila* with the highest levels of activity and AnCE mRNA occurring during pupation. The *AnCE* (*RACE)* mutant (l(2)34Eb) is pupal lethal and the lethality can be rescued by P-transformation with a genomic copy of *AnCE*, indicating a vital role for the enzyme during metamorphosis. In adult insects, the CNS, the reproductive tissues and the haemolymph have the highest levels of ACE activity. The localisation of ACE to neurosecretory cells in the insect brain is consistent with ACE having a neuropeptide processing role whereas haemolymph ACE might function to inactivate susceptible circulating peptide hormones. Insect gonads are both a source of regulatory peptides and the target for hormones controlling reproduction and therefore it is not surprising to find high levels of a processing enzyme in these tissues.